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肿瘤研究的新焦点——外体



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New focus in cancer research: exosomes

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[**Abstract**] Exosomes represent a class of cell-derived bilayered membrane-bound nanovesicles defined by size, surface protein and lipid composition, and the ability to carry bio-information. Their contained mRNAs, miRNAs and proteins are important mediators of intercellular communication. Thus, exosomes participate in many normal and pathological processes. They appear to be promising new tools for the clinical diagnostics and potentially for novel therapeutic strategies. More and more studies focus on the roles of exosomes in cancer development, metastasis, drug resistance, diagnosis and cancer treatments. This article reviewed the current evidence concerning exosome-based cancer researches, as well as the biogenesis of exosomes and their molecular compositions.

[**Key words**] exosomes; cancer; metastasis; therapy resistance

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外体(exosomes)是细胞分泌的不可溶的膜性囊泡中的一种,是由脂质双层膜包绕形成的扁平半球体,直径30~100 nm,密度1.13~1.21 g/mL[1-5]。由Johnstone等[3]于1987年在网织红细胞培养液中分离并命名,后来发现多种细胞均能以胞外分泌的方式释放外体,如肥大细胞、T淋巴细胞、B淋巴细胞、血小板、肠上皮细胞、星形胶质细胞、树突状细胞、生殖细胞以及肿瘤细胞等[6-8]。外体广泛存在于多种体液当中,如血液、淋巴液、尿液、羊水、唾液、脑脊液、腹水及精液[8-12]。外体含有多种蛋白质、脂类、DNA、mRNA、微小RNA(microRNA,miRNA)和非编码RNA[13-14]。因此,外体可以作为信使介导细胞间通讯,从而改变细胞的生理功能或疾病状态。这为研究恶性肿瘤的发生、发展、侵袭转移、治疗抵抗以及诊断治疗等方面提供新的焦点。

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1 外体来源及生物学特性

外体来源于细胞内的多囊泡体(multi-vesicular body,MVB),MVB是细胞的早期内体的膜向内逆出芽形成的许多小囊泡,将部分膜蛋白、脂质、蛋白及核酸等选择性包入囊泡中形成。MVB形成后有3个去向:

①直接被溶酶体降解;②重新进入高尔基体再循环;③与胞膜融合释放到胞外成为不可溶性膜性囊泡,外体是其中的一种,也是最主要的活性成分。关于外体的生物形成机制以及细胞如何将生物信息分选包裹进入外体,尚未完全阐明,目前明确参与外体形成和内容物分选的重要蛋白是内体分选转运蛋白复合体(endosomal sorting complex required for transport, ESCRT),ESCRT 由 4 个主要蛋白组成[15-16],分别为 ESCRT-0、ESCRT-Ⅰ、ESCRT-Ⅱ和 ESCRT-Ⅲ;外体的分泌则依赖于 RabGTPases 蛋白,包括 Rab27A、Rab27B、Rab11 和 Rab35[17-18];细胞内的 Ca^{2+} 浓度的、pH 和神经酰胺合成抑制剂能够影响外体的分泌[19-20]。

外体在透射电镜下,呈典型的"杯"或"碟"状,不同细胞来源的外体含有一些共同的蛋白质和脂质成分,如与膜转运、融合以及 MVB 形成相关的蛋白质 RabGTPases、CD9、flotillin、annexin、alix、TSG101 以及热休克蛋白(如 Hsp70 和 Hsp90)和溶酶体相关膜蛋白 2b(lysosome-associated membrane protein 2b, lamp2b)等[21];此外还都含有四跨膜蛋白(tetraspanins),如 CD63、CD81、CD9、CD82[11] 等。其中 CD63、CD81 和 CD9 已经是广泛用于鉴定外体的标志物,但潜在单一的、特异性的外体标志物还没有被发现[11]。由于来源细胞的多样性,外体也含有和来源细胞相关的蛋白成分,如 MHC 分子、协同刺激分子和肿瘤抗原等。这种多样性为外体发挥不同功能奠定了物质基础[22],目前已经鉴定超过 11 000 种蛋白与外体有关[23];外体还富含脂类,如胆固醇、鞘磷脂、磷脂酰丝氨酸和神经节苷脂,其中鞘磷脂和磷脂酰丝氨酸在质膜脂筏结构中起重作用[24];此外,外体还含有大量的核酸物质,其中 mRNA 在功能上可以被翻译成蛋白[2],miRNA 也同样具备调控相应靶基因的功能[25]。

2 外体与肿瘤侵袭转移

外体介导的细胞间通讯的可能机制有 4 种:①外体的膜蛋白与靶细胞上的受体相互作用,从而激活细胞内信号通路;②外体的膜与细胞膜融合,释放信号分子到靶细胞内;③外体被靶细胞内吞进入靶细胞,这种内吞可以是受体介导的特异性的,也可以是非特异性的,其机制是外体与受体细胞结合后,结合部位的细胞膜凹陷形成有被小窝,在网格蛋白复合物牵动下,有被小窝与质膜脱离进入细胞内,继而外体的膜与内体膜融合,释放信号分子;④外体的膜蛋白在细胞外被蛋白酶破坏,释放信号分子作为配体结合到靶细胞表面受体,激活靶细胞内的信号级联反应。外体可以通过上述的某种方式介导肿瘤细胞与微环境之间的对话,改变微环境细胞的功能,从而促进恶性肿瘤的发展和转移。

本课题组发现三阴性乳腺癌 MDA-MB-231 细胞特异性高表达 miR-105,通过外体转运到肿瘤基质和远端组织,被内皮细胞吞噬后,靶向降低内皮细胞中 miR-105 的靶基因——紧密连接蛋白的表达水平,致使血管内皮细胞紧密连接屏障遭到破坏,血管渗透性增强,从而促进三阴性乳腺肿瘤细胞的侵袭和转移[26];同时本课题组也发现来自于三阴性乳腺癌 MDA-MB-231 细胞的外体还可以转运 miR-122 到基质细胞,通过降低成纤维细胞和星形胶质细胞内靶蛋白丙酮酸激酶水平,降低基质细胞摄取和利用葡萄糖的能力,促使更多的葡萄糖供肿瘤细胞所利用,从而促进恶性肿瘤的发展和转移[27]。除此之外,本课题组的另一项研究结果显示乳腺癌细胞还可通过分泌的外体激活肿瘤基质巨噬细胞内的 NF-κB 信号通路,促进炎症因子的释放,促进肿瘤的发展[28]。同样,基质细胞也可以通过分泌外体影响肿瘤细胞:如成纤维细胞分泌的外体通过 Wnt-PCP 信号[Wnt-planar cell polarity (PCP) signaling]途径增强乳腺癌细胞的运动能力,促进乳腺癌的侵袭和迁移[29];IL-4 活化的巨噬细胞通过分泌含有高水平 miR-233 的外体促进乳腺癌细胞的侵袭[30]。

类似的结果也见于其他恶性肿瘤:Kucharzewska 等[31] 发现胶质母细胞瘤分泌的外体可将低氧调节相关基因的 mRNA 和蛋白转运到脑部健康细胞中,促使内皮细胞分泌一些强力的生长因子和细胞因子,激活 PI3K/Akt 信号通路,从而促进肿瘤的生长和迁移;Peinado 等[32] 报道了来自于高转移性黑素瘤的外体通过受体酪氨酸激酶持续地"驯化"骨髓祖细胞,导致大量地将分化为血管的骨髓祖细胞被招募到肿瘤转移部位,促进新生血管的生成。一项最新研究揭示,肿瘤细胞分泌的外体通过携带不同的整合素分子,帮助肿瘤细胞特异性地定向侵袭到不同的靶器官[5]。

3 外体与肿瘤治疗抵抗

肿瘤细胞对化疗药物和放疗的抵抗仍然是癌症治疗的主要障碍[33-34]。研究表明外体可通过不同机制介导肿瘤细胞的治疗抵抗。其中重要的一种机制是通过外体传递耐药相关的蛋白或基因:如顺铂抗性的卵巢癌细胞高表达铂类药物耐药相关的膜联蛋白 A3(annexin 3),并能通过外体将耐药表型传递给受体细胞[35]。Her-2 阳性的乳腺癌细胞系对曲妥珠单抗

(trastuzumab)的耐药性与分泌含有高水平 Her-2 的外体有关[36]。P-糖蛋白(P-glycoprotein, P-gp)是细胞膜上药物外排的关键分子,P-糖蛋白的高表达会导致肿瘤细胞对多种化疗药物的耐药。有研究表明,耐多西他赛的前列腺癌细胞分泌的外体与宿主细胞一样,含有高水平的多药耐药基因(multi-drug resistance gene 1, MDR-1)编码的 P-糖蛋白(MDR-1/P-gp),将其与敏感细胞共培养后,细胞对药物敏感性显著降低[37]。Bebawy 等[38]、Lv 等[39]采用荧光追踪技术,证明了耐药细胞来源的外体从宿主细胞表面获得了 P-gp,与敏感的受体细胞结合,可将 P-gp 运输至敏感细胞,诱导敏感细胞的耐药性。除了蛋白质,细胞也可以通过外体内的 miRNAs 传递耐药性,例如耐多西他赛的乳腺癌细胞株[40-41]。

另一种机制是外体参与了药物的泵出与进入:卵巢癌细胞能够借助外体的分泌将顺铂泵出细胞外[42-43],使细胞对顺铂产生耐药性。有研究者在肾癌模型中发现,含有 TGF-β1 的外体,在损伤的上皮细胞中激活成纤维细胞,从而导致结缔组织的增生[44-45],增强药物进入肿瘤细胞的屏障,增加肿瘤耐药性的产生。

除以上方式外,外体也可以通过多种信号途径介导肿瘤细胞的治疗抵抗:Wang 等[46]报道,骨髓基质细胞来源的外体能够阻断蛋白酶体抑制剂硼替佐米(bortezomib)引起的 Bcl-2 的下降,并使 Bcl-2 的水平升高,从而诱导多发性骨髓瘤细胞(multiple myeloma, MM)对蛋白酶体抑制剂硼替佐米的耐药。Boelens 等[47]报道了基质细胞分泌的外体转运非编码 RNA 和转座因子激活乳腺肿瘤细胞 RIG-I 信号途径,协同转录激活因子 STAT1,促进对 NOTCH3 转录反应,继而引起乳腺肿瘤细胞的放疗抵抗,阻断 NOTCH 信号通路可重新恢复放疗的敏感性。

4 外体在肿瘤诊断与治疗中的应用

4.1 外体与肿瘤诊断

由于外体携带有来源细胞特异性的 mRNA、蛋白质以及 miRNA 等成分,因此可以作为特异的生物学标志用于肿瘤和一些非肿瘤疾病的诊断,其优势在于循环中外体存在于包括血液在内的各种体液中,信息物质稳定性好、取材方便及易于跟踪检测,并可减轻患者的痛苦。例如通过鉴定外体中的核酸——即疾病特异性表皮细胞生长因子受体转录本已经被用来作为生物标记物诊断胶质母细胞瘤[27]。

我们发现在小鼠乳腺癌移植瘤模型预转移阶段和转移阶段,血清外体中 miR-105 和 miR-122 水平显著升高,发生转移的乳腺癌患者血清中外体内的 miR-105 和 miR-122 的水平也显著高于没有发生转移的患者,患者肿瘤组织中相应的靶基因水平显著降低,研究提示血清中的 miR-105 和 miR-122 很可能作为预测乳腺癌预后和转移的生物标志物[26-27]。同样,在卵巢癌患者血浆外体中 miRNAs 的表达谱发生明显改变,其中 miR-21、miR-141、miR-200a、miR-200b、miR-200c、miR-203、miR-205、miR-214 特异性高表达,且与肿瘤组织表达水平一致[48-49]。肺癌患者外周循环中外体中有 12 种 miRNAs 与肿瘤组织表达水平密切相关[50],胶质瘤患者的血清外体中也检测到胶质瘤特征性的 miRNAs[27]。

外体也携带有肿瘤相关的蛋白标志物,如黑色素瘤患者的外体中含有高浓度的 Caveolin-1 蛋白和 CD63[48-49];卵巢癌患者和肺癌患者血浆外体含有高水平上皮细胞黏附分子(EpCAM),且与肿瘤发展进程相关[48,50];外体携带的磷脂酰肌醇蛋白多糖-1(glypican-1, GPC-1)基因编码蛋白可以作为早期胰腺癌诊断的标志物[51];来自前列腺癌患者尿液中的外体含有异常高水平的前列腺特异性抗原(prostate specific antigen, PSA)、前列腺癌基因 3(prostate cancer gene 3, PCA3)、前列腺特异膜抗原(prostate specific membrane antigen,PSMA)和肿瘤相关标志 5T4[52]。Silva 等[53]研究发现结肠癌患者血浆中肿瘤来源外体显著增多,且癌胚抗原的表达含量很高,此类患者预后差、生存率低。有趣的是,对前列腺癌的外体中的脂质成分分析表明,某些脂质也可以作为候选生物标志物[54]。

4.2 外体与肿瘤治疗

肿瘤来源的外体携带有肿瘤特异性的抗原,如 Her-2 和 CEA,为肿瘤的免疫治疗提供了新的策略。已经证实树突细胞(dendritic cells, DC)来源的外体携带有 MHC 肽复合物和共刺激分子 CD86 等[55],能够增强 $CD8^+$T 细胞、$CD4^+$T 细胞活化,或将 MHC 抗原肽复合物转运给其他 DC,增强 DC 的抗原提呈能力[56]。因此,DC 分泌的外体可能替代 DC 发挥抗肿瘤的作用。目前此项研究处于Ⅱ期临床试验,用于无法手术治疗的非小细胞肺癌患者Ⅲ期与Ⅳ期的免疫维持治疗。受试者接受环磷酰胺化疗后,将自身的负载肿瘤抗原的外体进行回输,有效地诱导了机体的抗肿瘤免疫应答,使患者病情保持稳定[57-58];另一项临床研究结果显示,自体 DC 分泌的外体负载 MAGE3 抗原肽,用作疫苗治疗Ⅲ/Ⅳ期(转移性)黑色素瘤患者,经

皮下注射,显著升高 NK 细胞的数量[59-60]。自体腹水来源的外体(AEX)与粒细胞-巨噬细胞集落刺激因子(GM-CSF)联合治疗结直肠癌,显著增强了细胞毒 T 细胞(cytotoxic T cell,CTL)直接杀伤肿瘤细胞的作用[60]。以上研究证实外体可以在人体安全和有效地被传递,并且显示了在癌症治疗方面的广阔前景。

除了递送抗原,外体还可以递送药物[61-62]和 miRNAs[63-64],以达到靶向治疗肿瘤的目的。鉴于外体 在来源、大小和主要成分以及生物学功能上均不同于人工膜脂质体(liposomes),相比较脂质体化学转染法和显微注射等物理方法,外体作为细胞的天然产物,具有跨越生物屏障的能力强、毒性低、处理方便且易保存等优势[65-67]。利用外体运送 miRNA 或 siRNA 至受体细胞,能够降低受体细胞相关癌基因的表达,达到治疗或抑制肿瘤的目的。研究表明,将针对 RAD51 和 RAD52 的 siRNA 导入外体,递送到受体细胞,有效引起了 RAD51 和 RAD52 转录后的沉默,并诱导了大量处于增殖周期的肿瘤细胞的死亡[68-69]。类似研究还包括将 let-7a miRNA 导入外体,通过静脉注入表达表皮生长因子受体的小鼠乳腺癌组织,进行抗肿瘤治疗[70],以及将高水平表达自杀基因及其蛋白质的外体注射到小鼠神经鞘瘤模型中,此类基因或蛋白质在肿瘤细胞内可转换成抗癌剂,引发全身性的抗肿瘤治疗[71]。

也有研究通过减少或去除循环中外体来达到抑制肿瘤的发生与转移的目的,方法包括靶向微管组装和稳定系统、靶向外体分选分泌系统以及使用质子泵抑制剂[36,72-76]。美国圣地亚哥的 Aethlon 医学生物技术公司尝试使用体外过滤(Hemopurifier™)的方式去除晚期癌症患者血液中有害的外体,达到延缓疾病进程的目的。该公司还发明了 Her-2osome™减少循环中肿瘤细胞分泌的负载 Her-2 的外体,从而抑制 Her-2 阳性乳腺癌的发展[77]。

5 展望

综上所述,外体作为携带大量生物信息的膜性囊泡,是细胞间信号传递的重要信使,介导了肿瘤细胞与微环境细胞的通讯,通过不同的机制来影响肿瘤发展、侵袭转移和治疗抵抗,为肿瘤的基础研究开辟了一个全新的领域,同时在临床诊断和治疗方面也显示了巨大的应用潜力。人类对于外体的研究才刚刚开始,我们相信随着研究的不断深入,以及外体分离、纯化技术的改进,其生化特征、生物学功能将会逐渐明确。这将为我们阐明恶性肿瘤的发生、发展、侵袭转移以及治疗抵抗提供新的思路,也为恶性肿瘤的临床诊断、预防、预后和治疗提供科学依据,最终为人类征服恶性肿瘤发挥其应有的作用。

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